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SOIL MICROORGANISM POPULATIONS ON KAOLIN SPOIL WITH DIFFERENT VEGETATIVE COVERS

William J. Otrosina[1], Donald H. Marx[1] and Jack T. May[2]*

[1] *USDA Forest Service, Institute for Mycorrhizal Research and Development, Southeastern Forest Experiment Station, Forestry Sciences Laboratory, Carlton Street, Athens, GA 30602 (U.S.A.)*
[2] *School of Forest Resources, University of Georgia, Athens, GA (U.S.A.)*

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Abstract

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Keywords: bacterial populations; denitrification; fungal populations; kaolin spoils; nitrification; nitrogen fixation.

Nitrogen fixation and populations of fungi, nitrifying bacteria, denitrifying bacteria and general soil bacteria were estimated for kaolin spoils with differing vegetative covers. Spoils with herbaceous cover tended to have the highest nitrifying, denitrifying and other bacterial populations. Soil fungal populations on these herbaceous sites approached those on undisturbed pine forest sites. The fungal genera Aspergillus, Chaetomium, Verticillium *and* Fusarium *were more common on the herbaceous sites than on sites with other vegetative covers.* Oidiodendron *were isolated more frequently on spoils supporting pine species.*

Nitrogenase activity was higher on kaolin spoils with herbaceous cover than on bare spoils or those with 7-year-old planted pines. Nitrogenase activity was highest in undisturbed pine forest soil and in naturally revegetated kaolin spoil.

Further study of the effects of reclamation practices on microbial populations and processes involved in nutrient cycling on kaolin spoil is suggested by such populations on spoils with herbaceous cover.

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Introduction

Kaolin deposits occur in irregular pockets or lenses along the fall line in Georgia and South Carolina. Overlying strata are mixed during surface mining of this mineral, and the spoils are characterized by extremes in particle size and low nutrient content (May et al., 1972). Unlike spoils resulting from surface mining of coal or metallic ores, kaolin spoils have no toxic characteristics.

Because availability of inorganic nutrients to higher plants is mediated by activities of soil microorganisms, information on the soil microflora and their ecological roles on kaolin spoil is important for evaluation of procedures for reclaiming kaolin spoil. In a summary of many studies, Wilson (1965) described populations of fungi and bacteria and processes of ammonification, nitrification and carbon dioxide evolution for coal spoils in West Virginia and Pennsylvania. The study reported here was conducted to obtain an initial survey of the characteristics of microbial populations on kaolin spoils supporting different types of vegetation and to open new avenues of investigation on the microbiology of kaolin spoils.

Materials and methods

Description of research sites

Study sites were in Twiggs, Wilkinson and Washington counties in central Georgia. Among the 15 sites chosen were 3 unvegetated (bare) spoils,

TABLE I

Description of sites sampled for microbiological analyses

Site code	Site description
I	2-year-old bare spoil
II	10-year-old bare spoil
III	20-year-old bare spoil
IV	7-year-old slash pine plantation on spoil site 1
V	7-year-old slash pine plantation on spoil site 2
VI	7-year-old slash pine plantation on spoil site 3
VII	1-year-old spoil with herbaceous cover (lespedeza—fescue grass)
VIII	3-year-old spoil with herbaceous cover (lespedeza)
IX	4-year-old spoil with mixed grass cover (bahia, fescue, bermuda and lespedeza)
X	1-year-old spoil with herbaceous cover (rye and fescue grasses on 6—10 cm forest soil layer spread over spoil)
XI	40-year-old naturally revegetated spoil 1 (loblolly pine)
XII	40-year-old naturally revegetated spoil 2 (loblolly pine)
XIII	30-year-old undisturbed loblolly pine stand 1
XIV	30-year-old undisturbed loblolly pine stand 2
XV	30-year-old undisturbed loblolly pine stand 3

4 spoils artificially seeded to herbaceous cover, 3 spoils with 7-year-old slash pine (*Pinus elliottii* Engelm. var. *elliottii*) plantings, 2 spoils with 30-year-old natural loblolly pine (*P. taeda* L.) stands, and 3 undisturbed stands supporting 30-year-old loblolly pines. These sites represent a cross-section of artificially and naturally introduced vegetation on kaolin spoils in the region. Table I shows the age and type of vegetation on each site and provides a code number for each site.

Sampling procedure

Six 1-liter soil samples were randomly taken from each site during the spring or autumn of the same year. Litter was brushed away to expose mineral soil prior to sampling. Each sample was a composite of 5 subsamples taken with a trowel from a 10 m × 10 m plot to a depth of 10 cm. The sampling trowel was cleaned with 95% ethanol between samples. Soil samples were chilled in an ice chest and transported to the laboratory, where they were thoroughly mixed, screened through an 8-mm mesh screen to remove large stones and organic debris, and stored at 5°C. From each soil sample, 50-g portions were removed to gravimetrically determine moisture content by oven-drying to constant mass at 105°C. All microbiological procedures were performed 2—4 days after collection.

Fungal and bacterial populations

Populations of soil fungi in the spring and autumn samples were determined by the dilution plate method. Fifty g (ovendry basis) of each sample were serially diluted by factors of 10 in sterile distilled water and plated on rose bengal—streptomycin agar (Martin, 1950). Platings were replicated 3 times per dilution. Dilutions were one above and one below expected population range determined from preliminary trials. One ml of soil dilution was dispensed per plate and evenly spread over the agar surface with a sterile glass rod. The plates were incubated at 23°C. After 5 days, the plates within a dilution yielding colonies in the range 15—50 per plate were counted. Populations of fungi were expressed as colony forming units (CFU) per gram of oven-dried soil. Genera of fungi readily identified on the plates were recorded. Colonies which appeared frequently but were not readily identifiable were also recorded and then transferred to malt extract agar plates for later identification.

Because of constraints of time and working space, populations of soil bacteria were assayed only from the autumn samples. As with soil fungi, similarly bracketed decimal serial dilutions were used. Plating was on half-strength nutrient broth (Difco); 0.2 ml of soil dilution was placed on each of 4 plates per dilution. Incubation conditions were the same as above except that bacterial colonies were counted after 7 days. Populations were estimated on the dilution which yielded an average range of 20—200 colonies per plate, and expressed as CFU per gram of oven-dried soil. Occasional plates which were overgrown with motile bacteria were discarded, and populations were based on colony counts of the remaining plates in the dilution set. Analyses of variance were performed on all fungal and bacterial population parameters calculated from CFU counts. Population differences due to site were analyzed by Duncan's multiple range test (Steel and Torrie, 1960).

Nitrifying and denitrifying bacteria

Nitrifying and denitrifying bacteria were estimated from spring and autumn samples by the MPN method (Alexander, 1965). A 5 replicate by 5 decimal dilution (5× 5) series for both bacterial groups was used.

Screw cap test tubes containing 3 ml of medium were autoclaved (15 min at 121°C at 15 p.s.i.), cooled, inoculated with 1 ml of soil dilution, loosely capped, and incubated in the dark at 23°C on a laboratory bench. After 3 weeks, tubes were analyzed for nitrate with diphenylamine.

Denitrifying bacteria were estimated by the method of Focht and Joseph (1973). Incubation conditions were identical to those of the nitrification tubes, except that caps were tightened to restrict oxygen. Presence or absence of nitrate was also determined by diphenylamine.

Nitrogenase activity

Nitrogenase activity was determined for the autumn samples by the acetylene reduction technique (Hardy et al., 1968). For each sample, 25-g portions of soil were placed in each of three 50 ml Ehrlenmeyer flasks fitted with a 1.25 cm rubber serum stopper; 15 ml of the atmosphere in each flask were replaced with acetylene using a syringe. All sample flasks, along with 3 "acetylene only" and 1 "soil only" control flask from each sample, were incubated on a laboratory bench for 18 h at 25°C. Amount of ethylene produced was expressed as nanomoles of ethylene per gram of oven-dried soil per 24 h.

Three soil samples were taken during the autumn from each site and chemically analyzed for pH, total N, and available P, K and Ca. Nitrogen was determined by a microKjeldahl procedure (Institute of Ecology, University of Georgia, Athens). The remaining elements were assayed from sub-samples processed by double acid extraction and analyzed spectrophotometrically (Soil Testing and Plant Analysis Laboratory, Cooperative Extension Service, University of Georgia, Athens).

Results

Fungal populations

No significant differences between the spring and autumn samples were found, so the data for both seasons were combined. In general, undisturbed forest soil and kaolin spoils with seeded herbaceous covers had similar fungal population densities, but the naturally revegetated spoils and the 4-year-old lespedeza—fescue grass spoil had significantly higher fungal population densities than all other sites (Table II). Bare spoils and spoils with 7-year-old slash pine plantings had the lowest fungal populations. With the exception of two sites, fungal populations generally increased in the order: non-vegetated spoils $<$ 7-year-old pine planting $<$ undisturbed forest soil $\leqslant$ herbaceous covered spoil $\leqslant$ naturally revegetated spoil. The exceptions to this trend are an unexpectedly high fungal population on herbaceous site IX and a very low population on herbaceous site X.

The distribution and frequencies of certain fungal genera found in the kaolin spoil samples are presented in Table III. *Fusarium*, *Verticillium*, *Aspergillus* and *Chaetomium* tended to occur more frequently on spoils

TABLE II

Means and standard errors of fungal and bacterial populations on kaolin spoil sites with differing vegetative covers[1]

Site	Fungal populations in 10^3 CFU/g soil[2]	Bacterial populations in 10^6 CFU/g soil[3]
Spoil — bare		
I	0.75 ± 0.11 a	0.42 ± 0.08 a
II	0.26 ± 0.05 a	0.35 ± 0.10 a
III	0.42 ± 0.11 a	0.45 ± 0.12 a
Spoil — 7-year-old pine planting		
IV	38.00 ± 6.8 a	2.91 ± 0.88 a
V	38.60 ± 4.5 a	2.11 ± 0.50 a
VI	39.30 ± 6.6 a	2.12 ± 0.32 a
Spoil — herbaceous vegetation		
VII	163.20 ± 69.6 b	99.79 ± 12.52 c
VIII	199.40 ± 15.7 b	110.00 ± 16.93 c
IX	280.50 ± 12.9 c	73.95 ± 17.89 d
X	20.50 ± 1.4 a	17.64 ± 2.08 ab
Spoil — naturally revegetated		
XI	281.10 ± 21.5 c	11.50 ± 2.52 ab
XII	276.60 ± 19.3 c	9.45 ± 2.48 ab
Undisturbed pine stand		
XIII	198.40 ± 11.8 b	27.60 ± 7.98 b
XIV	175.30 ± 8.4 b	8.12 ± 0.76 ab
XV	196.10 ± 22.0 b	12.49 ± 2.53 ab

[1] Numbers with the same letter are not significantly different at $P \leqslant 0.05$.

[2] Means based on combined spring and autumn samples totaling 12.

[3] Means based on 6 samples taken in the autumn.

TABLE III

Distribution and frequencies of fungal genera in kaolin spoil sites under differing vegetative covers[1, 2]

Genera	Spoil — bare			Spoil — 7-yr-old pine planting			Spoil — herbaceous vegetation				Spoil — naturally revegetated		Undisturbed pine stand		
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
(Fungi imperfecti)															
Aspergillus	1	1	0	0	0	0	8	6	10	12	0	0	1	1	0
Cephalosporium	0	2	0	0	0	1	0	0	0	6	1	0	1	0	0
Chloridium	0	0	0	0	0	1	0	0	1	1	3	3	0	0	0
Cladosporium	8	9	0	3	5	2	6	4	0	3	0	1	1	0	0
Fusarium	0	0	0	0	0	0	10	10	12	12	2	1	1	0	0
Metarrhizum	0	0	0	0	0	0	0	0	0	0	3	1	1	0	2
Oidiodendron	0	1	0	12	12	12	0	0	0	0	7	4	1	1	0
Paecilomyces	1	1	0	0	3	0	0	1	3	2	2	0	3	3	2
Penicillium	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Pestalotia	0	2	0	0	0	0	4	0	0	0	3	1	1	0	0
Scolecobasidium	0	2	0	0	0	0	0	0	0	1	0	0	1	0	1
Trichoderma	1	1	1	5	11	12	12	6	10	2	12	10	7	12	9
Verticillium	0	0	0	0	0	0	0	4	8	2	2	1	1	0	0
(Phycomycetes)															
Absidia	0	0	0	0	0	1	0	2	0	0	6	4	0	5	4
Gongronella	0	0	0	5	0	4	0	4	3	0	1	0	0	2	0
Motierella	0	0	0	2	9	6	0	0	3	1	9	10	9	10	9
Mucor	0	0	0	0	6	6	0	8	4	2	2	1	1	6	5
Zygorhynchus	0	0	0	0	0	0	0	0	1	2	0	0	2	1	1
(Ascomycetes)															
Carpenteles	0	1	0	0	0	0	0	0	0	1	0	1	1	0	0
Chaetomium	0	0	0	0	0	3	4	2	1	2	0	0	0	0	0

[1] Zero (0) indicates fungus was not isolated from sample.

[2] Numerals 1—12 indicate the number of samples out of 12 in which fungus was isolated.

TABLE V

Soil chemical analysis (p.p.m.) of kaolin spoil sites selected for microbiological investigation

Site	pH	N	P	K	Ca	Mg
Spoil — bare						
I	5.1	50	4	24	60	20
II	5.0	100	4	24	60	20
III	5.0	60	4	24	63	20
Spoil — 7-year-old pine planting						
IV	4.7	410	4	24	60	20
V	4.4	300	4	24	60	20
VI	4.7	350	4	24	60	20
Spoil — herbaceous vegetation						
VII	6.7	500	12	24	412	44
VIII	6.6	1500	30	29	500	161
IX	4.9	970	17	37	264	102
X	7.1	700	19	37	480	151
Spoil — naturally revegetated						
XI	4.7	1800	4	24	76	20
XII	4.6	2900	4	24	96	20
Undisturbed pine stand						
XIII	4.4	1700	5	24	60	20
XIV	4.5	2800	4	24	86	20
XV	4.6	2200	4	24	60	20

values (VII, VIII and X). Total N was highest in the undisturbed forest site and naturally revegetated spoils.

Discussion

Fungal and bacterial populations

There is a positive influence of vegetation on microbial populations on kaolin spoils. Although coal spoil material is physically and chemically dissimilar to the material on kaolin spoils, the findings of Wilson and Stewart (1956) followed the same basic pattern reported here. The 5-fold increase in fungal population densities in the 7-year-old pine plantings over non-vegetated spoil sites reflects the capacity of kaolin spoil material to support fungal populations when litterfall and sloughed-off root material is available. The dramatically larger fungal and bacterial populations on sites with herbaceous cover compared to other sites examined in this study is reflected, in part, by the rapid increase in substrate afforded by the residues from grass and herbaceous plants. Fibrous root systems of grasses effectively distribute organic matter in the upper few centimeters of spoil, and the root systems, dead foliage and stems provide a relatively constant source of energy for both bacteria and fungi.

On Site IX, population densities of fungi and bacteria exceeded those in undisturbed forest soil. Wilson and Stewart (1956) also found higher populations of fungi and bacteria on coal spoils supporting grass cover than on undisturbed forest soils.

Amounts of inorganic soil nutrients such as N, P, K and Ca also influence growth of soil microorganisms (Cochrane, 1958). Since fertilizer containing these nutrients was added to kaolin spoils before seeding of herbaceous vegetation and pine planting, the microbial populations on these sites may reflect this improvement in pH and inorganic nutrition. Chemical analyses of spoil material from these sites revealed the highest levels of N, P, K and Ca of all spoil sites, with the exception of the relatively high N content in the naturally revegetated spoil (Table V).

Higher bacterial populations on the herbaceous sites reflect the increases in soil pH caused by lime application. Wilson (1965) also observed this effect of liming on bacterial populations in coal spoils in West Virginia.

In this study, it was difficult to separate vegetation or organic matter effects on fungal and bacterial populations from effects of inorganic nutrients added to the spoil for the establishment of vegetation. Because of N inputs, as well as other nutrients to the herbaceous covered sites, the higher populations of fungi and bacteria may reflect more favorable soil C:N ratios or simply more total substrate available to microbial attack. Such an effect has been observed by Wilson and Hedrick (1957), who found large increases in numbers of microorganisms on N-deficient coal spoils after the addition of organic matter in the form of alfalfa.

Somewhat anomalous results were observed on Site X, the herbaceous site on which a layer of forest soil had been spread prior to sowing with rye and fescue grasses. This site had much lower fungal and bacterial counts than the other herbaceous sites. The fungal counts on this site were lower (by a factor of 10) than those of the undisturbed forest soil investigated, even though the soil spread on Site X was similar to the undisturbed forest soil. Reasons for the low counts are not known.

Fungal genera

The dilution plate technique used to obtain these data in the present study isolates primarily those fungi which sporulate readily and are capable of growth on the isolation medium employed (Warcup, 1955). This technique precludes the isolation of Basidiomycetes, most Ascomycetes and certain Phycomycetes. However, relative changes in fungal generic composition have been detected.

The reported differences in frequencies of occurrence of fungal genera between undisturbed forest soil and herbaceous-covered kaolin spoil agree with the data of Miller et al. (1957) comparing forest soils and cultivated fields in Georgia. They found that *Penicillium* spp. and *Trichoderma* spp. were common in all soils, while *Fusarium* spp., *Aspergillus* spp. and the fungi of the Mucorales were more common in cultivated fields.

Thornton (1960) also found *Fusarium* spp. to be common in grassland soils and in forest soils converted to pastureland but rare in forest soils supporting arboreal vegetation. Another fungal genus, *Chaetomium*, found on kaolin spoils with herbaceous cover is interesting from the standpoint of its ecological role in cellulose decomposition. This genus was isolated from only one site supporting pine (Site VI). *Oidiodendron*, also a cellulose decomposer (Tribe, 1960), was common on all kaolin-spoil sites supporting pine.

The fungi belonging to the order Mucorales are represented in all sites having vegetation. These fungi are usually initial colonizers of new organic matter added to soil (Garrett, 1951), and their absence on bare spoil may reflect the lack of organic matter. The occurrence of *Mortierella*, the only genus in this group to be more common under pines than under herbaceous cover, agrees with the data of Thornton (1960), who found *Mortierella* to be more common in pine forest soils than in grassland soils. Unfortunately, the ecological roles of most soil fungi are not known and further research is needed to define these roles with respect to vegetation type and their influence on organic matter decomposition on kaolin spoil sites.

Nitrifying bacteria

The MPN technique employed to enumerate populations of nitrifying bacteria is inherently inaccurate (Alexander, 1965), especially when coupled with the variable nature of kaolin-spoil material. In spite of this problem, it still provides a useful means of determining general population trends among sites.

Nitrifying bacterial populations were virtually absent in all sites except those with herbaceous cover. Only one site yielded a statistically significant difference between spring and autumn samples, and this difference may be a sampling artifact. While sampling a grass-covered watershed in North Carolina, R.L. Todd (personal communication, 1975) found that nitrifying bacterial populations differed between winter and summer, but not between spring and autumn. Therefore, results of sampling on kaolin spoils may show different trends with different sampling times.

Kaolin spoil with herbaceous cover has the capacity to support nitrifying bacteria. Site VII, for example, had a high population of these bacteria during the first growing season after lespedeza and fescue were sown. Wilson and Stewart (1955) also noted rapid build-up of nitrifying bacteria in grass-vegetated coal spoils when ammonium sulfate was added and the pH of the spoil was neutralized by the addition of calcium hydroxide. It is generally held that nitrifying bacteria are favored by soil pH approaching neutrality (Alexander, 1961).

Low pH, N and P levels in bare spoil and in spoil beneath 7-year-old pines could explain the lack of nitrifying bacteria on those sites. The absence of nitrifiers in the naturally revegetated spoils and the undisturbed soil, each with relatively high total soil nitrogen levels, may have been caused by competition between higher vegetation and nitrifying bacteria for ammonium ions, low soil P concentrations (Purchase, 1974a, b), and nitrification-inhibiting phenolic compounds present in the litter on these sites (Rice and Pancholy, 1972, 1973, 1974).

Since nitrifying bacteria derive their energy solely from oxidation of ammonia to nitrate, the recovery of populations of these organisms from spoil samples implies that nitrification is an active process in kaolin spoils with herbaceous cover. Because of its mobility and acidic properties, the nitrate anion may contribute to loss of N and nutrient cations from these sites. Therefore, direct measurements of nitrate and nutrient cations leaching through spoil are needed on kaolin sites with herbaceous cover to assess the impact of nitrification on fertilizer nutrient retention and productivity of grass cover.

Denitrifying bacteria

Study procedures detected the presence of denitrifying bacteria, but the presence of these bacteria in soil does not necessarily mean that denitrification is taking place (Valera and Alexander, 1961).

Populations of these bacteria were highest on sites with herbaceous cover and were approximately proportional to the populations of nitrifying bacteria on these sites. Potentials for nitrification and denitrification exist on the same sites. McGarity and Meyers (1968) suggest that soluble organic materials serving as energy sources for denitrifying bacteria may leach down in the soil profile, together with nitrate, to sites of low oxygen concentrations. Respiring root systems and associated rhizosphere microorganisms may increase oxygen demand and create anaerobic microsites conducive to nitrate respiration (Stefanson, 1972). The lower soil pH on the other vegetated sites, particularly undisturbed pine forest soils and naturally revegetated spoil, may have been responsible for the lower bacterial counts from these sites (Valera and Alexander, 1961; Bremner and Shaw, 1968). The low denitrifying bacterial population found in bare spoils and the 7-year-old pine planting may also be due to low soil organic matter levels.

Nitrogenase activity

Although variation among samples was high, nitrogenase activity appeared to be related to vegetation type on kaolin spoil. Certain sites (III and XIV) had unusually high variations in nitrogenase activity among samples. The reason is not known. Low nitrogenase activity in bare kaolin spoils was expected.

Higher nitrogenase activity on kaolin spoil with herbaceous cover than on bare spoils and spoils with the 7-year-old pines probably reflects an interaction of several factors. Liming increases nitrogen fixation by reducing soil acidity (Moore, 1966). Jurgensen (1973) and Jurgensen and Davey (1970) point out that in addition to supplying calcium, liming may increase the availability of soil P and other nutrients necessary for supporting populations of *Azotobacter* (Jensen, 1965). Since lime has been applied to the kaolin sites with herbaceous cover, the vegetation may supply the necessary organic substrate for N-fixing microorganisms. Carbon-rich substrate can be supplied by sloughing of rhizosphere material (Rovira, 1965), while respiratory activities of plant roots and associated microorganisms can create anaerobic microsites suitable for development of *Clostridium* spp. (Jurgensen and Davey, 1970). Generally, conditions for potential denitrification and anaerobic N-fixation are similar (Stefanson, 1973) and probably exist on kaolin spoils with herbaceous cover. N-fixation on these sites may partially offset losses of N by denitrification.

General considerations

Kaolin spoils with herbaceous vegetation support higher microbial populations than spoils with other types of vegetation. The rapid build-up of organic matter by this vegetation is one explanation. Since all nutrient cycling and transfer to higher plants are mediated by microorganisms, and since microorganisms are nutrient sinks (Stark, 1973), any cultural practice that quickly establishes ecologically beneficial groups of these organisms is important for kaolin spoil reclamation. Further studies on soil organic matter formation, organic amendments and soil microbiological processes involved in nutrient cycling on kaolin spoils are indicated for the integration of cultural procedures needed for successful amelioration of these sites.

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